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Lentiviral Vectors

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3 HIV-1-Derived Vectors for Gene Therapy

To produce vector particles that are replication-defective, the *cis*-acting sequences must be segregated as much as possible from the *trans*-acting sequences in the producer cells. This is achieved by expressing the viral proteins from a construct that lacks *cis*-acting sequences (the "packaging construct"), and the transgene linked to the *cis*-acting sequences from a separate construct (the "transfer vector"). Only the latter will be packaged into particles and transferred to the target cell, where the lack of *trans*-acting proteins prevents any further spreading of the transfer vector. The potential biohazard in such a system is the possibility of recombination between the two constructs, either within the producer cell, or within heterozygous particles that contain RNAs from both constructs. In the case of the latter, recombination occurs during reverse transcription with high frequency (COFFIN et al. 1997). This could lead to the recreation of a single genome containing both *cis* and *trans*-acting sequences, and thus the occurrence of a replication-competent retrovirus (RCR). Such events are dependent upon residual *cis*-acting sequences in the packaging construct that allow it to be encapsidated, and on the extent of homology between the two constructs (COFFIN et al. 1997).

In the development of HIV-1-derived vector systems over the past few years, a number of steps have been taken to minimize the possibility of RCR formation, as well as improvements with regard to the potential pathogenic effects even if such an unlikely event should occur. These are described in the sections that follow.

3.1 Early HIV-1-Derived Vectors

Early HIV-1-derived vectors, intended not as gene delivery vehicles but as tools for the study of HIV-1 biology, consisted of nearly intact viral genomes containing disruptions or deletions in the *env* gene, and insertion of reporter gene cassettes in its place (PAGE et al. 1990; LANDAU et al. 1991). Either the HIV-1 *env* or a heterologous *env* was provided on a separate plasmid. However, such vectors could never be considered for therapeutic purposes, as the viral titres were low, and the risk of formation of RCR was high.

3.2 First Generation Vectors

In the first generation of HIV-1-derived lentiviral vectors that were intended for use as gene delivery vehicles, the structural genes were split between two plasmids, one expressing *gag*, *pol*, *tat* and *vif* under the control of the human cytomegalovirus (CMV) immediately early promoter, and the other expressing *env* and *rev* (PAROLIN et al. 1994). The packaging signal was deleted and the 3′ LTRs were replaced with the SV40 polyA signal. The transfer constructs contained the *cis*-acting sequences and the neomycin-resistance gene under control of the murine leukaemia virus (MLV) LTR promoter. However, titres were again low, and the vector could only infect a restricted range of target cells, the natural targets of HIV. A major improvement to lentiviral vectors came with the design of another 3-plasmid system by NALDINI et al. (1996b). This system became the prototype upon which almost all subsequent HIV-1-derived lentiviral vectors were based. In this case, the packaging construct contained the whole HIV-1 genome with the LTRs and packaging signal deleted, and the reading frame of Env was blocked. Expression was under the control of the CMV promoter in the place of the 5′ LTR. The 3′ LTR was replaced with the polyadenylation signal from the insulin gene. The *cis*-acting sequences required for packaging (ψ), reverse transcription (PBS), and integration (LTRs) of transcripts derived from this construct were therefore absent. It did, however, produce the structural proteins Gag and Gag-Pol, the regulatory proteins Tat and Rev, and the accessory proteins Vif, Vpr, Vpu, and Nef. A heterologous envelope was provided on a second plasmid, usually either the G glycoprotein of the vesicular stomatitis virus (VSV-G) or the MLV amphotropic envelope (Ampho). VSV-G binds to ubiquitous phospholipid components of the cell membrane, thus rendering the vector pantropic (BURNS et al. 1993). The VSV-G envelope also confers the particles high stability, allowing them to be stored for extended periods, and/or concentrated by ultracentrifugation (BARTZ and VODICKA 1997). The third plasmid is the transfer vector, which contains all of the *cis*-acting sequences of HIV required for transcription of the viral genome (LTRs, RRE), packaging (ψ, 350bp of *gag*), reverse transcription (PBS), and integration (LTRs), as well as the gene of interest under the control of an appropriate promoter (see Fig. 2A). Transcription and transport to the cytoplasm of full-length RNAs could therefore only occur in the presence of Tat and Rev, which are provided in *trans* by the packaging construct in producer cells, and which are absent from target cells. Particles were generated by transfecting all three of these constructs into 293T human kidney cells and collecting the cell supernatant approximately 40h later. In this way, titres of 10^5 or higher were obtained with the Ampho or VSV-G envelope. This compares favourably with the titres of MLV-based vectors produced in the same way (NALDINI et al. 1996b).

These vectors were then tested for their ability to infect various cell types, including cells blocked at various stages of the cell cycle. They were found to efficiently transduce cell lines that were cycling or arrested in G_1-S or G_2, as well as, but to a lesser extent, cells in G_0. Non-proliferating primary human macrophages were also significantly transduced, as were terminally differentiated neurons in the brains of rats directly injected with concentrated virus preparations (NALDINI et al. 1996a,b).

A slight modification of this system was then made, in which most of the *env* gene was deleted, leaving only the RRE sequence of *env* in the packaging transcripts. For a schematic representation of this first generation packaging construct, see Fig. 1B. This system was tested in a variety of primary cell types, and found to be efficient for the in vivo transduction and long-term gene expression in adult neurons (NALDINI et al. 1996a; BLOMER et al. 1997), photoreceptor cells in the retina (MIYOSHI et al. 1997), muscle cells, and liver cells (KAFRI et al. 1997).

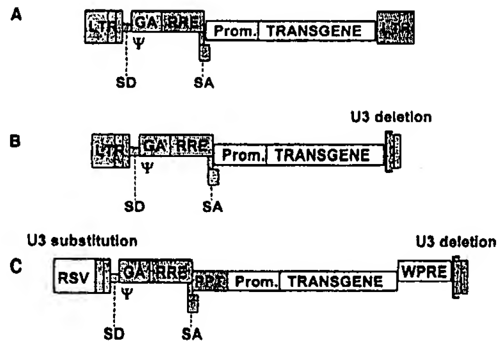

Fig. 2A–C. Transfer vectors. All HIV-1-derived sequences are shaded in *grey*, and all non-HIV-1 sequences are in *white*. These vectors contain the *cis*-acting sequences required for RNA processing, packaging, reverse transcription, and integration into target cell DNA. **A** An LTR transfer vector, with the wild-type HIV-1 LTRs maintained, plus about 300 bases of *gag*, and the RRE. The transgene of interest under the control of a heterologous promoter is inserted. **B** A SIN vector, with the U3 region of the 3′ LTR deleted. Upon reverse transcription in the target cell, the U3 LTR will be copied to the upstream position, resulting in an inactivated 5′ LTR promoter upon integration. **C** An improved SIN vector, with the 5′ LTR U3 region replaced by a strong constitutive promoter from the Rous sarcoma virus, to allow expression of the viral genome in the absence of Tat. The HIV-1 central PPT has been inserted 5′ to the internal promoter, and the WPRE has been inserted 3′ of the transgene. *RSV*, Rous sarcoma virus promoter; *Prom*, internal promoter; *WPRE*, woodchuck hepatitis virus post-transcriptional regulatory element. Only the relevant portions of the constructs are shown

In terms of biosafety, the first generation of HIV-derived lentiviral vectors were replication defective. The formation of RCRs is unlikely due to the use of a 3-plasmid system and a heterologous envelope, as well as to the removal of multiple *cis*-acting sequences from the packaging construct. Most importantly, the lack of most of the HIV *env* sequence from the packaging system made it impossible for the regeneration of wild-type HIV-1 from any type or number of recombination events that might occur during vector production. In tests for the presence of RCR, none were detected (see references above).

3.3 Second Generation Vectors

In spite of the extremely low probability that the first generation system could regenerate a RCR with a non-lentiviral envelope, the possibility could not be formally dismissed. Furthermore, the packaging construct still expressed all of the HIV-1 proteins with the exception of Env. Some of these proteins are essential virulence factors for HIV-1, and have been shown to elicit potentially detrimental cellular responses. For example, Vpr causes cell cycle arrest, Vif can inhibit growth in some cell types, and Nef can induce apoptosis (Federico 1999). Considering that Nef, Vif, and Vpr can be incorporated into viral particles and delivered to the target cell, a much higher degree of biosafety would be achieved if they could be deleted

from the system. Zufferey et al. (1997) and Kim et al. (1998) describe packaging constructs that are deleted in some or all of *vif*, *vpr*, *vpu* and *nef*. The efficiency of viral particle production was not significantly affected, even with all four accessory genes eliminated. These vectors also retained the ability to transduce non-dividing cell lines in vitro and mature rat neurons in vivo in the absence of all accessory proteins (Kafri et al. 1997; Zufferey et al. 1997). For a schematic representation of a second generation packaging construct, see Fig. 1C.

The ability to remove the accessory genes can be at least partially explained by looking at the function of these genes in the wild-type HIV-1 life cycle. Vif is only required during HIV-1 assembly in cells which have a "non-permissive" phenotype. 293T cells, in which virus particles are produced, are "permissive", and thus Vif is not required for infectious particle assembly. The role of Vpu in the HIV-1 life cycle is also dispensable in the vector production system. Vpu down-regulates CD4 expression in the ER to prevent interaction with HIV-1 Env. However, the vectors have a heterologous Env, and are produced in cells which do not express CD4. Vpu is also believed to stimulate the release of virions from the cell, but this effect was not observed in 293 or 293T cells. As with Vpu, the CD4-downregulating activity of Nef would obviously not be required in this system. However, Nef also functions to promote the infectivity HIV-1 virions. The lack of a requirement for Nef in the second generation system may be explained by the use of the VSV-G envelope. Aiken (1997) showed that pseudotyping of HIV-1 with VSV-G, which changes the mechanism of target cell entry to an endocytic pathway, rather than direct fusion with the plasma membrane, markedly suppressed the requirement for Nef. Finally, the lack of a requirement for Vpr in most cell types can be explained by the redundancy of nuclear import signals present in the PIC, as described previously.

All of the accessory genes that have been removed from the vector production system are genes that play essential roles in the life cycle and virulence of wild-type HIV-1. Thus, even in the unlikely event of multiple recombination events leading to the formation of a RCR, the pathogenic properties of the parental virus would not be reconstituted.

3.4 Third Generation and SIN Vectors

In the second generation system, the *tat* and *rev* genes remained in the packaging construct along with *gag* and *pol*. Tat plays a crucial role in HIV-1 pathogenesis, as its powerful transcriptional activation drives the exceedingly high rate of viral replication in vivo. Moreover, Tat has been implicated in the development of Kaposi's sarcoma, in addition to inducing a number of other potentially detrimental cellular responses (Federico 1999; Bartz and Emerman 1999). Thus the ability to remove *tat* from the packaging construct was a further improvement in biosafety of the packaging system. This is possible if the U3 region of the 5′ LTR in the transfer vector construct is replaced by constitutively active promoter sequences, such as the CMV promoter, whereupon Tat becomes dispensable and can be deleted from the packaging construct (see Fig. 2C) (Kim et al. 1998; Dull et al.

1998). In addition, it was shown that Rev could be provided on a separate plasmid, making the expression of the packaging functions conditional on complementation available only in producer cells (Dull et al. 1998). The possibility of eliminating the requirement for Rev entirely was also investigated. This was done by replacing the RRE with other RNA transport elements derived from either the Mason-Pfeizer monkey virus (MPMV) or the hepatitis B virus (HBV) (Gasmi et al. 1999). These elements rely on endogenous factors within the host cell, and thus would bypass the need for Rev, or any other exogenous factor, for RNA transport to the cytoplasm. However, when these elements were tested for their ability to substitute for Rev and RRE, the HBV element did not function, and the MPMV element led to a five- to tenfold decrease in retroviral particle production (Gasmi et al. 1999). Alternatively, the Rev requirement for Gag-Pol expression could be relieved by the recoding of the genes to eliminate inhibitory sequences which prevent expression, and inserting the new genes into an expression vector (Schneider et al. 1997; Valentin et al. 1997; zur Megede et al. 2000). It should be noted, however, that Rev is still required to express the transfer vector RNA and cannot be eliminated from the packaging system. For a schematic representation of a third generation packaging construct, see Fig. 1D.

The removal of *tat* and the increased subdivision of the remaining HIV-1 sequences into four plasmids instead of three, makes the formation of a replication competent and pathogenic virus even more unlikely than with earlier systems. However, one concern remaining in terms of biosafety was that the transfer vector retained the ability for transcription of the full-length genome after integration into the target cell, albeit at a low level due to the lack of Tat. It would, however, be possible for the vector to be mobilized by replication competent virus (for example, if the transduced cell were subsequently infected with wild-type HIV-1). In addition, there is the possibility that the 3′ LTR could induce aberrant expression of adjacent genes. For these reasons, vectors with self-inactivating (SIN) LTRs were designed. This was achieved by creating a deletion in the U3 region of the 3′ LTR. During reverse transcription in the target cell, this deletion is transferred to the 5′ LTR of the proviral DNA. If the deletion is sufficient to abolish the transcriptional activity of the LTR promoter, the transcription of full-length vector RNA would be eliminated in transduced cells. The possibility of insertional activation of adjacent cellular oncogenes would also be reduced. Furthermore, as there would be no complete U3 sequence in the producer system, recombination to regenerate a wild-type U3 would not be possible.

Miyoshi et al. (1998) performed a 133-bp deletion in the U3 region of the 3′ LTR which removed the TATA box and binding sites for Sp1 and NF-B, resulting in transcriptional inactivation of the proviral LTR in infected cells, both in vitro and in vivo. There was no decrease in transcripts in producer cells, and no significant reduction in viral titre. The expression of the transgene in vivo in both brain neurons and retinal cells was improved with SIN vectors, perhaps due to the removal of transcriptional interference by the HIV-1 LTR promoter (Miyoshi et al. 1998). Zufferey et al. (1998) were able to achieve a similar result with deletion of up to 400bp of the 3′ LTR U3 region. Again, virus particle production was not

decreased, nor was transduction efficiency in vitro or in vivo. Furthermore, Bukovsky et al. (1999) demonstrated that in SIN vector transduced cells that were subsequently infected with wild-type HIV-1, the vector was not mobilized. Transfer vectors with SIN LTRs are illustrated in Fig. 2.

In addition to improved biosafety, the use of SIN vectors has two added advantages: elimination of transcriptional interference by the LTR promoter, and the possibility to create tissue-specific and inducible vectors, which would be difficult in the presence of non-specific transcription from the LTR promoter.

Even with the advanced third generation design, it is impossible to entirely eliminate the possibility of homologous recombination events, as there are sequences that need to be present in both the packaging and the transfer vectors: 40–300bp of *gag*, which are required for efficient packaging of the transfer vector, and the RRE, which is required in both constructs for transport of transcripts to the cytoplasm. Several recent approaches to eliminate or decrease this residual overlap have resulted in moderate losses in vector titre as compared to the vectors described above (A. Bukovsky et al., unpublished data). However, most of these vector systems need to be tested in challenging applications in vivo in order to accurately evaluate their performance. It should be noted that recombination events between transfer and packaging constructs that lead to the restoration of the *gag* and *pol* genes to the transfer vector allow RCR monitoring by validated and sensitive assays based on HIV-1 Gag detection. The formation of such a recombinant, however, would produce a construct that, upon integration into target cells, would have no promoter to drive expression of the *gag* and *pol* genes due to the SIN nature of the 3′ LTR in the transfer vector. Furthermore, transport of transcripts to the cytoplasm would be Rev-dependent, and thus would not occur in Rev-minus target cells. Therefore the predicted biosafety of this system is very high. See Fig. 3 for an illustration of the entire third generation vector production system.

3.5 Recent Advances

In addition to improving biosafety of lentiviral vectors, modifications have also been made to improve the efficiency of gene delivery to and expression in target cells. One of these modifications involved the insertion of the post-transcriptional regulatory element from the genome of the woodchuck hepatitis virus (WPRE) at the 3′ end of the transfer vector (see Fig. 2C). The WPRE acts at the post-transcriptional level, by promoting nuclear export of transcripts and/or by increasing the efficiency of polyadenylation of the nascent transcript (Zufferey et al. 1999; Vigna and Naldini 2000), thus increasing the total amount of mRNA in cells. The addition of the WPRE to lentiviral vectors resulted in a substantial improvement in the level of transgene expression from several different promoters, both in vitro and in vivo (Zufferey et al. 1999; Deglon et al. 2000; Consiglio et al. 2001).

As described previously, there is a central PPT located within the *pol* gene of wild-type HIV-1 that results in a 100-nucleotide DNA flap in the viral DNA of the

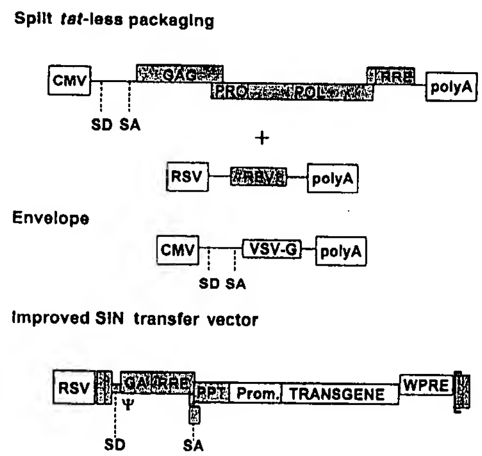

Fig. 3. Cotransfection of four constructs is required for the production of third generation HIV-1-derived lentivirus vectors. Shown here is the entire 4-plasmid system, consisting of the split *tat*-less packaging constructs, the heterologous envelope (which is shown here to be the vesicular stomatitis virus G-glycoprotein, VSV-G, but which could also be other heterologous envelopes such as the murine leukaemia virus amphotropic envelope), and the improved SIN transfer vector. Upon transfection of all four of these constructs into human 293T cells, high titres of replication-defective, self-inactivating vectors are produced

PIC, which has been implicated in the nuclear transport of both HIV-1 and lentiviral vector PICs (Zennou et al. 2000; Follenzi et al. 2000). When the 118-bp cPPT sequence was restored to late generation SIN vectors (Fig. 2C), the transduction efficiency was significantly increased in several types of primary cells, both growing and growth-arrested, indicating that nuclear import is a rate-limiting step in the transduction of dividing and non-dividing cells (Follenzi et al. 2000).

3.6 HIV-2-Derived Lentiviral Vectors

Lentiviral vectors based on HIV-2 are also being developed. HIV-2 is less pathogenic than HIV-1 in humans (Kanki et al. 1994; Marlink et al. 1994), and therefore could be more acceptable for deriving vectors for clinical purposes. The feasibility of an HIV-2-based vector has recently been demonstrated by Arya et al. (1998) and Poeschla et al. (1998). The latter were VSV-G pseudotyped and were able to efficiently transduce human T and monocytoid cell lines, growth-arrested HeLa cells, terminally differentiated human macrophages, and NTN2 neurons. It has also been demonstrated that HIV-2-derived vectors can be cross-packaged with HIV-1 packaging functions (Poeschla et al. 1996; Corbeau et al. 1998). However, in all cases the packaging systems used were equivalent to the early generation of HIV-1 vectors; the accessory and regulatory genes were all present in the packaging constructs, and the transfer vectors contained fully active HIV-2 LTRs. The dispensability of the accessory genes and the ability to use SIN vectors in the HIV-2-derived vector system remain to be demonstrated. Furthermore, some aspects of the HIV-2 viral genome and its life cycle are less well understood as compared to HIV-1. For instance, significant differences were demonstrated in the packaging signal of HIV-2 vs. HIV-1 (Poeschla et al. 1998) and the cross-encapsidation between the two viruses is not reciprocal, or as efficient. A better understanding of these aspects is required to advance HIV-2 vectors.

3.7 Stable Packaging Cell Lines

The establishment of a stable packaging cell line producing a high-titre lentiviral vector would be a significant improvement. The cell line could be fully characterized and it would also greatly facilitate upscaling of viral production for clinical purposes. The complexity of the system, combined with the cellular toxicity of the VSV-G protein, has made this a difficult task. However, significant progress has been made, as is described elsewhere in this volume.

4 Applications and Performance of HIV-1-Derived Lentiviral Vectors

Lentiviral vectors have the potential to deliver genes in a broad range of disease settings, including immune and metabolic deficiencies, neurodegenerative disease, viral infection, and cancer. Some of the important target cell types, as well as some specific disease models, in which lentiviral vectors have been efficacious are described briefly below.

4.1 Central Nervous System

As described previously, efficient in vivo transduction of the neurons of adult rodent brains was observed with all generations of vectors (Naldini et al. 1996b; Naldini 1998; Blomer et al. 1997; Zufferey et al. 1997, 1998; Miyoshi et al. 1998), and gene transfer to brain neurons of non-human primates has also been observed (Kordower et al. 1999). The promising applications of lentiviral vectors in the CNS are reviewed more extensively elsewhere in this volume.

4.2 Haematopoietic Stem Cells

Haematopoietic stem cells (HSC) are important targets for gene therapy, due to the ease with which they can be manipulated ex vivo and returned to the host, as well as the broad range of diseases that could potentially be treated in this way. The largely quiescent nature of HSC, combined with the need for vector integration to ensure gene delivery to the HSC progeny, makes them prime candi-

dates for lentiviral vector transduction. Successful transduction of primitive human cord blood and bone-marrow-derived NOD/SCID repopulating cells, using a short-term exposure to the vector in the absence of cytokines, has been shown with both early generation (Miyoshi et al. 1999) and late generation (Guenechea et al. 2000; Follenzi et al. 2000) HIV-1-derived vectors. In the latter report, very high levels of gene marking were obtained with the improved vector described above (Follenzi et al. 2000). Again, a review of the use of lentiviral vectors for the transduction of this important target cell type is presented elsewhere in this volume.

4.3 Lymphocytes

Resting T lympocytes are resistant to infection with both wild-type HIV-1 and HIV-1-derived vectors. These cells are infected by the virus, but fail to allow completion of reverse transcription (Zack et al. 1990, 1992). As most peripheral T lymphocytes are in G_0, this presents a significant obstacle to gene therapy strategies involving T lymphocytes, which could include therapy for immunodeficiencies, as well as immunotherapy approaches to the treatment of cancer. Korin and Zack (1998) demonstrated that progression to the G_1b phase of the cell cycle is required for completion of wild-type HIV-1 reverse transcription in T cells. This progression requires both activation of the cell through the T-cell receptor (TCR), as well as costimulation through a costimulatory receptor such as CD28. Activation of the TCR alone allows progression to G_1a, whereupon the cell cycle is arrested and the cell becomes subsequently impervious to further stimulation. In this state, as in the G_0 state, infection is non-productive (Korin and Zack 1998). If resting cells are treated with exogenous nucleosides, the efficiency of reverse transcription is improved, but there remains a blockage of productive infection in these cells, indicating that the inhibition occurs at multiple stages of the viral life cycle (Korin and Zack 1999). Artificially introducing the transcription factor NFATc, which is normally found in activated T cells, allowed productive infection with HIV without triggering proliferation (Kinoshita et al. 1998). This suggests that partial activation of target cells, without full cell-cycle progression, is sufficient for lentiviral infection of T lymphocytes. This activation induces the production of a limited number of proteins or other factors which then allow productive HIV-1 infection to occur, and which are presumably naturally present in other terminally differentiated cell types that can be transduced by lentiviral vectors despite their non-proliferative status (Emerman 2000).

In the case of HIV-1-derived vectors, in addition to highly efficient transduction of fully activated T-lymphocytes (Unutmaz et al. 1999; Costello et al. 2000; Chinnasamy et al. 2000), it appears that a significant level of transduction can be obtained with only partial activation by various cytokines (Unutmaz et al. 1999). The optimal transduction conditions and subsequent effects on T-cell function are still under investigation.

4.4 Disease Models

Long-term therapeutic efficacy of lentivirus mediated gene transfer into the CNS has been reported in murine models of retinal photoreceptor degeneration (Takahashi et al. 1999), type VII mucopolysaccharidosis (Bosch et al. 2000), metachromatic leukodystrophy (Consiglio et al. 2001), and Parkinson's disease (Bensadoun et al. 2000). More recently, Kordower et al. (2000) delivered lentiviral vectors expressing glial cell line-derived neurotrophic factor (GDNF) to the brains of aged monkeys or monkeys previously injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The latter induces a Parkinson's disease-like phenotype. Long-term gene expression was seen, as well as a reversal of functional deficits and a prevention of nigrostriatal degeneration.

In addition to the models of CNS disease described above, there are a few other disease models in which HIV-1-derived lentiviral vectors have been tested for their ability to function and possibly reverse the phenotype of the disease. Some of these are listed below.

Gallichan et al. (1998) showed that β-islet cells isolated from NOD/SCID mice and transduced with a lentiviral vector expressing IL-4, then implanted into diabetes-prone mice, provided protection from autoimmune insulitis and islet destruction.

May et al. (2000) showed that the human β-globin gene together with large segments of its locus control region could be transferred into murine bone marrow cells, which were then transplanted into lethally irradiated recipients. Human β-globin was found in up to 13% of total haemoglobin in normal recipients, and in 17–24% of the haemoglobin in β-thalassemic heterozygous recipients. The latter is a level which could provide therapeutic benefit in thalassemic patients, and indeed, there was a correction of the phenotype in these mice.

Follenzi et al. (2000) showed that expression of therapeutic levels of human factor IX (FIX) was obtained in the peripheral blood of SCID mice injected intravenously with lentiviral vectors expressing the FIX gene under the control of a CMV promoter. Park et al. (2000) were also able to obtain therapeutic levels of FVIII and FIX expression upon injection of HIV-1-derived vectors into the hepatic portal vein of mice.

5 Concluding Remarks

Many health disorders are potential targets for gene therapy, and lentiviral vectors, due to their ability to transduce non-dividing cells and stably integrate into the genome, provide a highly promising means for achieving such therapies. As described in this review, significant advances in vector design have led to highly improved vector safety, and the concern for the formation of a pathogenic, replication competent virus during vector production or target cell infection has been

virtually eliminated. Early experiments with HIV-1-derived vectors in animal models of disease, as discussed above, have yielded promising results. In addition, HIV-1-derived vectors have already proved to be extremely useful tools for the study of basic cellular biology and disease, as they provide efficient means of adding exogenous sequences to cells and observing the effects in both in vitro and in vivo models.

Further challenges are to develop methods for transcriptional targeting and regulation of therapeutic genes, or tissue specific targeting of lentiviral particles through manipulations of the Envelope proteins. The former has already been demonstrated with oncoretroviral vectors, where, upon transduction of $CD34^+$ haematopoietic cells, transcription was restricted either to the erythroid progeny or the megakaryocytic progeny by using the appropriate tissue-specific sequences in the transgene promoter (Wilcox et al. 1999; Grande et al. 1999). Furthermore, before considering the clinical application of these vectors to human patients, their safety must be proved through the use of reliable and sensitive assays and animal models, and a stable packaging cell line with a high vector output should be established. These challenges are already well on their way to being met.

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Lentiviral Vectors Derived from Simian Immunodeficiency Virus

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1 Introduction

The ultimate success of gene therapy to cure inherited or acquired genetic diseases relies on the development and on the availability of gene transfer vectors that can efficiently deliver a transgene following their administration in vivo. Several challenging hurdles need to be overcome to reach such a goal. A first prerequisite is that methods that allow the preparation of vectors at high titers and in culture systems with potential for large scale-up need to be optimized (Andreadis et al. 1999; Kotani et al. 1994; Smith et al. 1996). Second, the gene transfer vectors should not be recognized by the host immune system in order to avoid their inactivation (Cosset et al. 1995b; DePolo et al. 1999). Upon their delivery into gene therapy recipients, vectors should also be able to circumvent the numerous biological barriers that are likely to limit their diffusion and bio-distribution in the target organism. They should therefore be able to specifically recognize, penetrate and express the transgene in cells of the gene therapy target tissue (Diaz et al. 1998; Jager et al. 1999; Russell and Cosset 1999). Third, they should be able to replicate and to express a transgene in cells that are not proliferating or are slowly proliferating, a predominant situation in vivo. Last, but not least, they should be accepted by both ethical and regulatory authorities. In this respect the development of vectors derived from viruses that are not pathogenic to humans may be preferred.

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